

Laboratory Practices for Stool-Specimen Culture for Bacterial Pathogens, Including *Escherichia coli* O157:H7, in the FoodNet Sites, 1995–2000

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In 2000, we surveyed microbiologists in 388 clinical laboratories, which tested an estimated 339,000 stool specimens in 1999, about laboratory methods and policies for the routine testing of stool specimens for *Salmonella*, *Shigella*, *Campylobacter*, and *Vibrio* species, *Yersinia enterocolitica*, and *Escherichia coli* O157:H7. The results were compared with those of similar surveys conducted in 1995 and 1997. Although these laboratories reported routinely testing for *Salmonella*, *Shigella*, and *Campylobacter* species, only 57% routinely tested for *E. coli* O157:H7, 50% for *Y. enterocolitica*, and 50% for *Vibrio* species. The mean proportions of stool specimens that yielded these pathogens were as follows: *Campylobacter*, 1.3% of specimens; *Salmonella*, 0.9%; *Shigella*, 0.4%; and *E. coli* O157:H7, 0.3%. The proportion of laboratories that routinely tested for *E. coli* O157:H7 increased from 59% in 1995 to 68% in 2000; however, the proportion of stool specimens tested decreased from 53% to 46%. *E. coli* O157:H7 should be routinely sought in stool specimens submitted for microbiologic culture.

Clinical microbiology laboratories are the foundation of laboratory-based public health surveillance for infectious diseases [1, 2]. Through the identification and notification of culture-confirmed infections to public health authorities, clinical laboratories play a vital role in the recognition of infectious disease outbreaks and the ep-

idemiological understanding of disease trends over time [3, 4]. The interpretation of trends in laboratory-based surveillance data must therefore consider, among other things, laboratory testing procedures.

In 1996, the Foodborne Diseases Active Surveillance Network (FoodNet) began conducting active surveillance for laboratory-confirmed illness caused by bacterial pathogens that are commonly transmitted by food [5]. One goal of this network is to monitor more precisely the burden of foodborne illnesses in the United States. A key finding of FoodNet surveillance is that there are substantial variations in the incidence of laboratory-confirmed infection with bacterial foodborne pathogens between the different FoodNet surveillance areas (also known as "FoodNet sites") [6]. In 2000, for example, the incidence of *Escherichia coli* O157:H7 infection ranged from 0.4 cases per 100,000 population

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in Maryland to 4.8 in Minnesota, and the incidence of *Campylobacter* infection ranged from 7.0 in Maryland to 31.7 in California [6]. To describe culture practices used at clinical microbiology laboratories in the FoodNet sites and to determine whether variations in the incidence of laboratory-confirmed bacterial foodborne infection were due to laboratory practice, we conducted a survey of laboratory practices in 2000 and compared our results with those of previous surveys conducted in 1995 and 1997. These data, in combination with information about the frequency of pathogen isolation, were used to review the potential consequence of stool-specimen testing recommendations for *E. coli* O157:H7 testing.

METHODS

FoodNet investigators conducted active surveillance for laboratory-confirmed infections with *Salmonella*, *Shigella*, *Campylobacter* and *Vibrio* species, *E. coli* O157:H7 and other Shiga toxin-producing *E. coli* (STEC), and *Yersinia enterocolitica* in clinical laboratories located in FoodNet sites and at large commercial laboratories outside the sites that received stool specimens from residents of the sites. The FoodNet surveillance case definition excludes multiple isolations of the same pathogen from a patient within 30 days. Surveys of practices in laboratories participating in FoodNet surveillance were conducted in 1995, 1997, and 2000. In 1995, the FoodNet sites included Minnesota, Oregon, and selected counties in California (Alameda, Contra Costa, and San Francisco), Connecticut (Hartford and New Haven), and Georgia (Clayton, Cobb, DeKalb, Fulton, Gwinnett, Newton, and Rockdale); the total population of the 1995 FoodNet sites was 14.3 million persons (5.4% of the US population). In 1997, counties in Connecticut (Fairfield), Georgia (Barrow, Bartow, Carroll, Cherokee, Coweta, Fayette, Forsyth, Henry, Paulding, Pickens, Spaulding, and Walton), Maryland (Anne Arundel, Baltimore, Baltimore City, Carroll, Harford, and Howard), and New York (Albany, Columbia, Genesee, Greene, Livingston, Monroe, Montgomery, Ontario, Orleans, Rensselaer, Saratoga, Schenectady, Schoharie, Wayne, and Yates) were added; the population, according to 1997 postcensus estimates, was 16.1 million persons (6.6% of the US population). In 2000, counties in Colorado (Adams, Arapahoe, Denver, Douglas, and Jefferson) and Tennessee (Cheatham, Davidson, Dickson, Hamilton, Knox, Robertson, Rutherford, Shelby, Sumner, Williamson, and Wilson) were added; the total population in the 9 sites was 37.8 million persons (12.2% of the US population). We obtained appropriate informed consent from all participants and conducted the study in accordance with guidelines for human research as specified by the US Department of Health and Human Services.

A questionnaire designed to elicit descriptions of laboratory practices for the identification of *Salmonella*, *Shigella*, *Cam-*

pylobacter and *Vibrio* species, *E. coli* O157:H7 and other STEC, and *Y. enterocolitica* in stool specimens was distributed to the microbiology supervisors at all of the laboratories that participated in FoodNet surveillance. Data collected included information on routine testing practices and methods for the identification of the 6 bacterial pathogens under surveillance in FoodNet. The number of stool specimens tested during a time period before the survey was conducted was also collected. In the 1995 survey, the microbiologists were asked to report the number of stool specimens received in August 1995; in the 1997 and 2000 surveys, microbiologists were to report the number of stool specimens received during the previous year. We defined on-site testing for *E. coli* O157:H7 as performance of bacterial stool culture with sorbitol-MacConkey (SMAC) or cefixime-tellurite SMAC (CT-SMAC) agar or with an immunoassay for either Shiga toxin or for the O157 antigen, with a confirmation test performed on site or at the state public health laboratory.

We estimated the rate of isolation using the number of stool specimens that yielded *Salmonella*, *Shigella*, *Campylobacter*, or *E. coli* O157:H7 isolates and were identified through FoodNet surveillance in either 1996 or 1999, divided by the total number of stool specimens tested for each laboratory that reported routinely testing for the pathogen in that year.

To describe trends in practice, the subset of laboratories that were included in all 3 surveys were analyzed. To assess changes in practices, the frequency at which laboratories routinely tested all stool specimens for each pathogen and the number of stool specimens received were evaluated. Responses were entered into Epi-Info software, version 6.04 (Centers for Disease Control and Prevention, Atlanta, GA) and analyzed using SAS software, version 8.0 (SAS Institute).

RESULTS

Laboratory practices in 2000. A questionnaire was sent to all 436 laboratories that participated in active surveillance in the 9 FoodNet sites. Of these, 393 questionnaires (90%) were returned; 5 of the returned questionnaires were excluded from analysis because of missing data, leaving 388. The median reported time for completion of the questionnaire was 45 min (range, 3–300 min). An estimate of the number of stool specimens processed for bacterial pathogens in 1999, the year before the survey, was provided by 361 (93%) of the laboratories (table 1). Overall, the median number of stool specimens processed per laboratory in that year was 431 (range, 13–17,210 specimens).

Salmonella. Three hundred eighty-six (99%) of 388 participating laboratories reported testing stool specimens for *Salmonella* species. Of these, 368 (95%) tested on site. All laboratories that tested on site routinely tested all stool specimens

Table 1. Frequency and rate of the testing of stool specimens at clinical laboratories that reported the total number of stool specimens tested in 1999 FoodNet laboratory survey.

State	No. of laboratories		No. of specimens	
	That were surveyed	That reported the no. of specimens tested	Tested, median (range)	Total
California	23	20	645 (177–2250)	16,622
Colorado	15	15	831 (150–10,638)	29,565
Connecticut	36	33	774 (13–17,210)	44,898
Georgia	92	80	387 (15–9534)	56,671
Maryland	19	16	461 (125–13,800)	27,955
Minnesota	80	78	220 (22–10,600)	65,960
New York	26	25	872 (49–4097)	31,754
Oregon	58	56	275 (13–5060)	32,696
Tennessee	39	38	586 (92–3829)	32,585
All sites	388	361	431 (13–17,210)	338,706

for *Salmonella* species. These laboratories tested an estimated 330,771 stool specimens for *Salmonella* species in 1999.

Shigella. Three hundred eighty-six (99%) of 388 participating laboratories reported testing stool specimens for *Shigella* species. Of these, 367 (95%) tested on site. All laboratories that tested on site routinely tested all stool specimens for *Shigella* species. These laboratories tested an estimated 329,643 stool specimens for *Shigella* species in 1999.

Campylobacter. Three hundred eighty-one (98%) of 388 participating laboratories reported testing stool specimens for *Campylobacter* species. Of these, 356 (93%) laboratories tested on site. Of the laboratories that tested on site, 344 (97%) routinely tested all stool specimens for *Campylobacter* species. These laboratories routinely tested 312,206 (96%) of 325,336 stool specimens in the 356 laboratories that tested on site for *Campylobacter* species. Only 1 of the laboratories that routinely tested all stool specimens for *Campylobacter* species reported using a nonculture method (the Prospect *Campylobacter* Microplate Assay).

E. coli O157:H7. Three hundred sixty-seven (95%) of 388 participating laboratories reported testing stool specimens for *E. coli* O157:H7, and 209 (57%) routinely tested all stool specimens. Of the 158 laboratories that did not routinely test all stool specimens, 152 (96%) tested on physician request and 101 (64%) tested if the specimen appeared to be bloody. Taken together, 310 (84%) of 367 laboratories routinely tested at least all bloody stool specimens for *E. coli* O157:H7.

Of the 367 laboratories that reported testing for *E. coli* O157:H7, 293 (80%) tested on site; 272 (93%) used SMAC agar, 15 (5%) used CT-SMAC agar, and 2 (<1%) used both SMAC and CT-SMAC, methods that take advantage of the fact that *E. coli* O157:H7, unlike most other types of *E. coli*, does not ferment

sorbitol. The remaining 4 laboratories used only nonculture methods: 3 used a Shiga toxin immunoassay and 1 used immunoassays for both Shiga toxin and the O157 antigen; all 4 of these laboratories routinely sent Shiga toxin–positive specimens to the state public health laboratory for isolation and serotyping of STEC. Of laboratories that used SMAC or CT-SMAC, 8 (3%) also used an immunoassay for the O157 antigen and 6 (2%) also used a Shiga toxin immunoassay. In total, 18 (6%) of 293 laboratories that tested on site used a nonculture method to test for *E. coli* O157:H7.

Of the 289 laboratories that tested for *E. coli* O157:H7 on site using culture methods, 196 (68%) routinely tested all stool specimens for *E. coli* O157:H7. These 196 laboratories tested an estimated 150,161 (58%) of the 257,017 specimens received by the 289 laboratories that tested on site for *E. coli* O157:H7 using a culture method. One of 3 approaches was used after sorbitol fermentation–negative colonies were detected. First, 52 (18%) conducted complete on-site testing of stool specimens for *E. coli* O157:H7, including testing for agglutination to the O157 lipopolysaccharide (LPS), biochemical confirmation that the isolate was *E. coli*, and on-site H antigen testing. Twenty-three (44%) of 52 laboratories that conducted complete on-site testing also forwarded the isolate to the state public health laboratory or reference laboratory. Second, 159 (55%) laboratories conducted less than complete testing on site but also forwarded the isolate to the state public health laboratory or reference laboratory. Specifically, 51 laboratories (32%) tested for agglutination to the O157 LPS and biochemically confirmed that the isolate was *E. coli* on site, 15 laboratories (9%) tested for agglutination to the O157 LPS only, 63 laboratories (40%) biochemically confirmed that the isolate was *E. coli* on site only, and 30 laboratories (19%) conducted no additional testing of

sorbitol fermentation–negative colonies before forwarding the isolate to the state public health laboratory or reference laboratory. Third, the remaining 78 (27%) laboratories did not forward the isolate to a reference laboratory after the detection of sorbitol-negative colonies. Seventy (90%) of 78 laboratories that did not forward the isolate that was tested for agglutination to the O157 LPS on site. To increase the sensitivity of stool culture, 10 (3%) of 289 laboratories that tested for *E. coli* O157:H7 on site using culture methods used enrichment broth.

Non-O157 STEC. In total, 11 (3%) of 388 laboratories used a Shiga toxin immunoassay to screen for STEC, including non-O157 serotypes; these laboratories processed 45,080 (13%) of 338,706 total stool specimens tested in the FoodNet sites. Only 1 of 11 laboratories that tested for STEC using a Shiga toxin immunoassay tested all stool specimens for STEC using this test; this laboratory processed only 919 specimens in 1999. Of the 11 laboratories that tested some stool specimens for STEC using the Shiga toxin immunoassay, 8 (73%) facilitated the further characterization of Shiga toxin–positive stool specimens. Specifically, 3 laboratories cultured Shiga toxin–positive specimens for *E. coli* O157:H7 and sent specimens that did not yield *E. coli* O157:H7 to the state public health laboratory, and 5 laboratories sent the Shiga toxin–positive specimens to the state public health laboratory without further testing. The 3 laboratories that did not facilitate the further characterization of Shiga toxin–positive stool specimens included a large commercial laboratory that processed 10,600 stool specimens in 1999.

Yersinia. Three hundred twenty-eight (85%) of 388 participating laboratories reported testing stool specimens for *Yersinia* species. Of these, 280 (85%) tested on site. Of the laboratories that tested on site, 178 (64%) used cefsulodin-irgasan novobiocin (CIN) agar or *Yersinia*-selective agar. Of the 280 laboratories that tested on site, 139 (50%) routinely tested all stool specimens for *Yersinia*. These 139 laboratories tested an estimated 121,272 (40%) of 303,180 stool specimens received by laboratories that tested on site for *Yersinia* species in 1999. However, only 82 (59%) of 139 laboratories reported using CIN agar or *Yersinia*-selective agar. Of the 57 laboratories that routinely tested for *Yersinia* but did not use CIN agar, 33 laboratories used MacConkey agar, 18 used both MacConkey and xylose lysine deoxycholate (XLD) agar, 2 used XLD agar, and the remaining 4 laboratories did not indicate the selective media used.

Vibrio. Two hundred seventy-six (71%) of 388 participating laboratories reported testing stool specimens for *Vibrio* species. Of these, 212 (77%) tested on site; 113 (53%) used thiosulfate citrate bile salts sucrose (TCBS) agar, and 105 (50%) routinely tested all stool specimens for *Vibrio* species. These 105 laboratories tested an estimated 116,302 (51%) of the 228,043 stool specimens received in 212 laboratories that tested

on site for *Vibrio* species in 1999. However, only 28 (27%) of 105 laboratories reported using TCBS agar. Of the 77 laboratories that routinely tested for *Vibrio* species but did not use TCBS agar, 68 laboratories used blood plate agar, 4 used MacConkey agar, and the remaining 5 laboratories did not indicate the selective media used.

Comparison with 1995 and 1997 surveys. A total of 160 laboratories in California ($n = 15$), Connecticut ($n = 17$), Georgia ($n = 17$), Minnesota ($n = 59$), and Oregon ($n = 52$) participated in each of the surveys that assessed laboratory practices in 1995, 1997, and 2000. Of these, 137 (86%) were hospital-based and 33 (14%) were independent laboratories, including group physician-practice laboratories and large commercial laboratories. Approximately two-thirds of the stool specimens processed by these 160 laboratories were obtained from outpatients (median proportion per laboratory, 69%). Of the 160 laboratories, 154 (96%) provided estimates of the number of stool specimens received in 1996 and 1999. The 154 laboratories reported receiving 111,271 stool specimens in 1996 (median number per laboratory, 321; range, 7–9429) and 125,630 stool specimens in 1999 (median number per laboratory, 320; range, 13–17,210).

In 1996 and 1999, the most commonly isolated pathogen was *Campylobacter* (1.4% and 1.2% of isolates, respectively; table 2). In general, rates of isolation of *Campylobacter* were higher in California and Oregon than in Connecticut, Georgia, and Minnesota. *Salmonella* was the next most commonly isolated pathogen (0.8% of isolates in 1996 and 0.9% in 1999). In general, *Shigella* was isolated at a higher rate than was *E. coli* O157:H7, except in Minnesota in 1996 and in Oregon and Connecticut in 1999. Between 1996 and 1999, the overall rate of isolation declined for *Campylobacter* and *Shigella*, increased slightly for *Salmonella*, and remained the same for *E. coli* O157:H7.

In this subset of laboratories surveyed in 1995, 1997, and 2000, the number of laboratories that routinely tested stool specimens for *Salmonella*, *Shigella*, and *Campylobacter* species remained constant between 1995 and 2000. In contrast, the number of laboratories that routinely tested all stool specimens for *E. coli* O157:H7 increased from 94 (59%) in 1995 to 108 (68%) in 2000. However, the proportion of stool specimens that were routinely tested for *E. coli* O157:H7 declined from 53% to 46% (table 3). Twenty-two (14%) of 160 laboratories began routinely testing for *E. coli* O157:H7 between 1995 and 2000, and 6 (4%) stopped routinely testing for *E. coli* O157:H7; the latter included a large commercial laboratory in Connecticut that processed 17,210 stool specimens in 1999. In January 1999, this laboratory began to test stool specimens for *E. coli* O157:H7 using culture methods only on physician request and then switched to a Shiga toxin immunoassay only on physician request in November 1999. Variations in testing practices occurred by site, with a higher proportion of laboratories in

Table 2. Rates of isolation among laboratories that reported routinely testing for *Salmonella*, *Shigella*, *Campylobacter*, or *Escherichia coli* O157:H7 during 1996 or 1999 by pathogen and FoodNet site.

FoodNet site	Mean rate of isolation (%) ^a							
	<i>Salmonella</i>		<i>Shigella</i>		<i>Campylobacter</i>		<i>E. coli</i> O157:H7	
	1996	1999	1996	1999	1996	1999	1996	1999
California	0.94	0.48	1.05	0.37	3.28	1.18	0.25	0.16
Connecticut	0.61	0.95	0.13	0.14	0.75	1.10	0.12	0.35
Georgia	0.61	1.48	0.85	0.47	0.58	0.97	0.04	0.07
Minnesota	1.16	0.83	0.31	0.41	1.60	1.14	0.41	0.25
Oregon	1.04	1.04	0.41	0.24	2.23	1.67	0.31	0.25
All sites	0.84	0.91	0.57	0.31	1.43	1.21	0.25	0.25

^a Mean of the reported no. of specimens cultured that yielded the pathogen and were identified through FoodNet surveillance divided by the total no. of stool specimens tested at the laboratories in the site.

Connecticut, Minnesota, and Oregon routinely testing stool specimens for *E. coli* O157:H7 than laboratories in California and Georgia (table 3). The proportion of laboratories that tested at least all bloody stool specimens (either routinely or when the stool specimen appeared bloody) increased from 84% in 1995 to 90% in 2000; however, the proportion of stool specimens that were tested in these laboratories declined from 93% to 78% (table 3).

The routine testing of stool specimens for *Yersinia* species was performed less frequently than testing for *Salmonella*, *Shigella*, and *Campylobacter* species. Of the 45 laboratories that routinely tested all stool specimens for *Yersinia* species in 1995, there were 18 laboratories that had stopped routinely testing by 2000, and 10 others began routinely testing by 2000. The routine testing of stool specimens for *Vibrio* species was least commonly performed. Of the 27 laboratories that routinely tested all stool specimens for *Vibrio* in 1995, there were 24 that had stopped routinely testing by 2001, and 12 others began routinely testing by 2000.

DISCUSSION

We found that almost all of the clinical laboratories that process stool specimens in the FoodNet sites routinely test all stool specimens for *Salmonella*, *Shigella*, and *Campylobacter* species. It is therefore unlikely that regional differences in the incidence of culture-confirmed illness caused by *Salmonella*, *Shigella*, or *Campylobacter* infection is related to laboratory culturing practice. Additional studies are needed to explain the regional differences, particularly with respect to *Campylobacter* infection. Variations in laboratory practice by site may explain some of the differences in the incidence of culture-confirmed *E. coli* O157:H7 infection. A high proportion of laboratories in Oregon and Minnesota routinely tested for *E. coli* O157:H7 infections; these sites had the highest incidence of *E. coli* O157:H7 infections. However, a study at 10 US hospitals conducted

between 1990 and 1992 found geographic variation in the rate of isolation of *E. coli* O157:H7, despite provision of resources so that all stool specimens were tested [7].

Because public health surveillance for foodborne diseases relies on clinical microbiology laboratory confirmation, it is encouraging that a high proportion of FoodNet laboratories tested at least all bloody stool specimens for *E. coli* O157:H7. Unfortunately, although the proportion of laboratories that reported testing at least all bloody stool specimens increased across the 3 surveys, the proportion of all stool specimens tested declined. In 1993, the Association of State and Territorial Public Health Laboratory Directors (later renamed the Association of Public Health Laboratories) recommended that clinical laboratories test at least all bloody stool specimens for *E. coli* O157:H7, in part on the basis of the high risk of severe complications or death and the public health imperative to investigate and prevent disease transmission [8]. However, the ascertainment of whether diarrhea is bloody cannot always be made by examining a stool specimen, and few laboratories receive this information from patients or clinicians. The difficulty in implementing stool culture policies on the basis of the presence or absence of blood in stool specimens, combined with the relatively high isolation proportion of *E. coli* O157:H7 calculated in the present study, supports a Centers for Disease Control and Prevention (CDC) recommendation that all stool specimens submitted for microbiological culture be tested for *E. coli* O157:H7 [9]. This recommendation is consistent with a 1994 consensus statement on *E. coli* O157:H7 made by a multidisciplinary panel in the United States [10] and recent guidelines published in the United Kingdom [11]. The isolation of *E. coli* O157:H7 from ill persons is the first critical step in the detection and investigation of outbreaks [12]. The molecular subtyping of *E. coli* O157:H7 isolates using PFGE as part of the CDC PulseNet program is a powerful tool in the surveillance and epidemiological characterization of different strains [13, 14]. Therefore, clinical microbiologists are strongly en-

Table 3. Testing practices for *Escherichia coli* O157:H7 at 160 clinical laboratories that participated in the 1995, 1997, and 2000 FoodNet laboratory surveys.

Testing practice, FoodNet site	1995		1997		2000	
	No. (%) of laboratories with practice ^a	No. (%) of stool specimens tested	No. (%) of laboratories with practice ^a	No. (%) of stool specimens tested	No. (%) of laboratories with practice ^a	No. (%) of stool specimens tested
Routine testing of all stool specimens						
California	4 (27)	323 (9)	4 (27)	3572 (18)	5 (33)	4079 (14)
Connecticut	8 (47)	1915 (66)	9 (53)	16,483 (80)	8 (49)	5904 (19)
Georgia	6 (35)	1043 (36)	7 (41)	10,064 (38)	7 (41)	5611 (29)
Minnesota	37 (63)	3101 (85)	42 (71)	20,797 (67)	44 (75)	32,636 (81)
Oregon	39 (75)	2032 (73)	40 (77)	18,665 (64)	44 (85)	21,107 (66)
All sites	94 (59)	8414 (53)	102 (64)	69,581 (55)	108 (68)	69,337 (46)
Routine testing of at least all bloody stool specimens						
California	11 (73)	3173 (88)	12 (80)	16,002 (82)	14 (93)	29,104 (98)
Connecticut	16 (94)	2902 (99)	16 (94)	20,531 (99)	15 (88)	12,983 (42)
Georgia	13 (77)	2535 (86)	15 (88)	19,102 (72)	15 (88)	14,123 (74)
Minnesota	45 (76)	3426 (94)	47 (80)	22,195 (71)	49 (83)	33,564 (83)
Oregon	50 (96)	2699 (97)	51 (98)	28,886 (99)	51 (98)	29,475 (92)
All sites	135 (84)	14,735 (93)	141 (88)	106,716 (84)	144 (90)	119,249 (78)

NOTE. Only the 154 laboratories that estimated the no. of stool specimens tested in August 1995 ($n = 15,881$) and annually in 1996 ($n = 126,936$) and 1999 ($n = 152,237$) are included.

^a No. (%) of laboratories in the FoodNet site that followed the specified practice for routine testing for *E. coli* O157:H7 on site.

couraged to forward *E. coli* O157:H7 isolates to their state public health laboratory as part of routine public health surveillance.

Testing for non-O157 STEC was uncommon among laboratories participating in FoodNet surveillance, despite the recognized role of these organisms in diarrheal disease and hemolytic uremic syndrome [15]. Furthermore, 3 laboratories, including 1 large commercial laboratory, did not forward specimens that were positive for Shiga toxin to the state public health laboratory for the isolation and serotyping of STEC isolates. The recovery of *E. coli* isolates in Shiga toxin–positive specimens is crucial to identify both O157 and non-O157 STEC in laboratories that use nonculture methods exclusively. The serotyping of *E. coli* isolates from Shiga toxin–positive specimens is also necessary to determine the burden of non-O157 STEC serotypes in the United States and to identify outbreaks of non-O157 STEC infection. Therefore, all specimens, broths, or isolates that are positive for Shiga toxin should be forwarded to the state public health laboratory for confirmation and STEC serotyping [9]. In addition, clusters of STEC-positive stool specimens should be reported to public health authorities for investigation.

Between 1996 and 1999 in the 5 original FoodNet sites, the annual incidence of culture-confirmed *Campylobacter* infection decreased from 23.5 cases/100,000 population to 17.5, that of *Shigella* infection decreased from 8.9 to 5.0, and that of *E. coli*

O157:H7 infection decreased from 2.7 to 2.1 [16]. The decline in the incidence of *Campylobacter* and *Shigella* infections was reflected by the declining rate of isolation during the same time period. This decrease in the rate of isolation provides some evidence of a true decline in incidence. If the decline in the incidence of culture-confirmed infection was due to other factors, such as a decline in the rate of physician testing of stool specimens, the corresponding changes in isolation rates may not have been observed. Changes in the methods or frequency of culturing do not explain the observed trends in FoodNet surveillance for *Salmonella*, *Shigella*, or *Campylobacter* infection. However, the stable rate of isolation of *E. coli* O157:H7 suggests that part of the reduction in the incidence of culture-confirmed infection between 1996 and 1999 may be attributed to a reduction in the proportion of stool specimens routinely tested for this pathogen.

The results of the 2000 FoodNet laboratory survey are similar to those of a nationwide survey of 601 clinical microbiology laboratories conducted by the College of American Pathologists in 1994 [17]. According to the results of that survey, most laboratories (>96%) tested routinely for *Salmonella*, *Shigella*, and *Campylobacter* species. In contrast, 47% of laboratories routinely tested for *Yersinia* species, 34% routinely tested for *E. coli* O157:H7, and 30% routinely tested for *Vibrio* species. A smaller survey of 67 clinical laboratories showed that 54% of laboratories tested for *Yersinia* species, 24% routinely tested

for *E. coli* O157:H7, and only 12% routinely tested for *Vibrio* species [18]. Boyce et al. [19] found that 29% of a random sample of 129 US laboratories surveyed in December 1994 and January 1995 routinely tested for *E. coli* O157:H7, with an increasing trend since 1985. In addition, laboratories in the western and northeastern United States were more likely to test for *E. coli* O157:H7 routinely and in bloody stool specimens, compared with laboratories in the midwestern and southern United States [19]. These findings of regional differences in laboratory practice are similar to the results of the FoodNet laboratory survey. Results from a survey of 102 laboratories in the Gulf states, a region with an increased incidence of non-cholera *Vibrio* infection, showed that routine culture for *Vibrio* using TCBS agar was performed at 20% of laboratories and for 22% of stool specimens [20]. This proportion is slightly lower than the 27% of laboratories in FoodNet surveillance that reported routinely testing for *Vibrio* using TCBS agar.

One limitation of the present analysis is the difficulty of verifying the estimate of the numbers of stool specimens processed. Large commercial laboratories located within the FoodNet surveillance areas may also have received specimens from outside the surveillance areas, which would increase the estimates of specimens tested and lead to lower calculated isolation rates. The estimates provided may also include multiple specimens from the same patient. However, the number of stool specimens tested was used to weight the relative volume of testing in laboratories. A more precise measurement of the number of stool specimens tested would be particularly useful in determining a more precise rate of isolation of *E. coli* O157 and facilitate the further evaluation of the stool culturing guidelines for this pathogen. Validation studies using computerized laboratory records or studies testing all stool specimens for pathogens in FoodNet surveillance conducted in sentinel laboratories could further clarify these issues.

Bacterial stool culture is one of the most labor-intensive and costly diagnostic tests, per culture-positive specimen [21–23]. To increase the proportion of stool specimens that yield a positive result (i.e., to reduce unnecessary testing), it has been suggested that routine culture of stool specimens from inpatients who have been hospitalized for >3 days should be rejected [18, 24, 25]. Targeted testing using epidemiological data may also increase the yield of bacterial stool culture. For example, for a patient with gastroenteritis who reports a recent history of raw seafood consumption, the inclusion of TCBS agar to test the stool specimen for *Vibrio* species is appropriate [20]. Health care providers should be aware of the bacterial, parasitic, and viral pathogens that are routinely tested in submitted stool specimens and aware of the public health significance of positive laboratory findings. An analysis showed that physicians in FoodNet sites in which the incidence of *E. coli* O157:H7 infection was lower were more likely to incorrectly assume that

screening for this pathogen was included in a routine bacterial stool culture [26]. Finally, several investigators have suggested that changes in the health care financing and cost-cutting measures in clinical microbiology laboratories may have a negative effect on the sensitivity of public health surveillance systems [27, 28].

Recent guidelines from the Infectious Diseases Society of America have included proposals intended to maximize the utility of bacterial stool culture for physicians, microbiologists, and public health officials who are interested in the surveillance and control of foodborne and diarrheal diseases [29]. These guidelines reiterate the previous recommendation that at least all bloody stool specimens should be tested for *E. coli* O157:H7. The relatively high rate of isolation of *E. coli* O157:H7 demonstrated in the present study, however, supports the recommendation that all stool specimens from patients with acute diarrhea be tested for *E. coli* O157:H7.

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